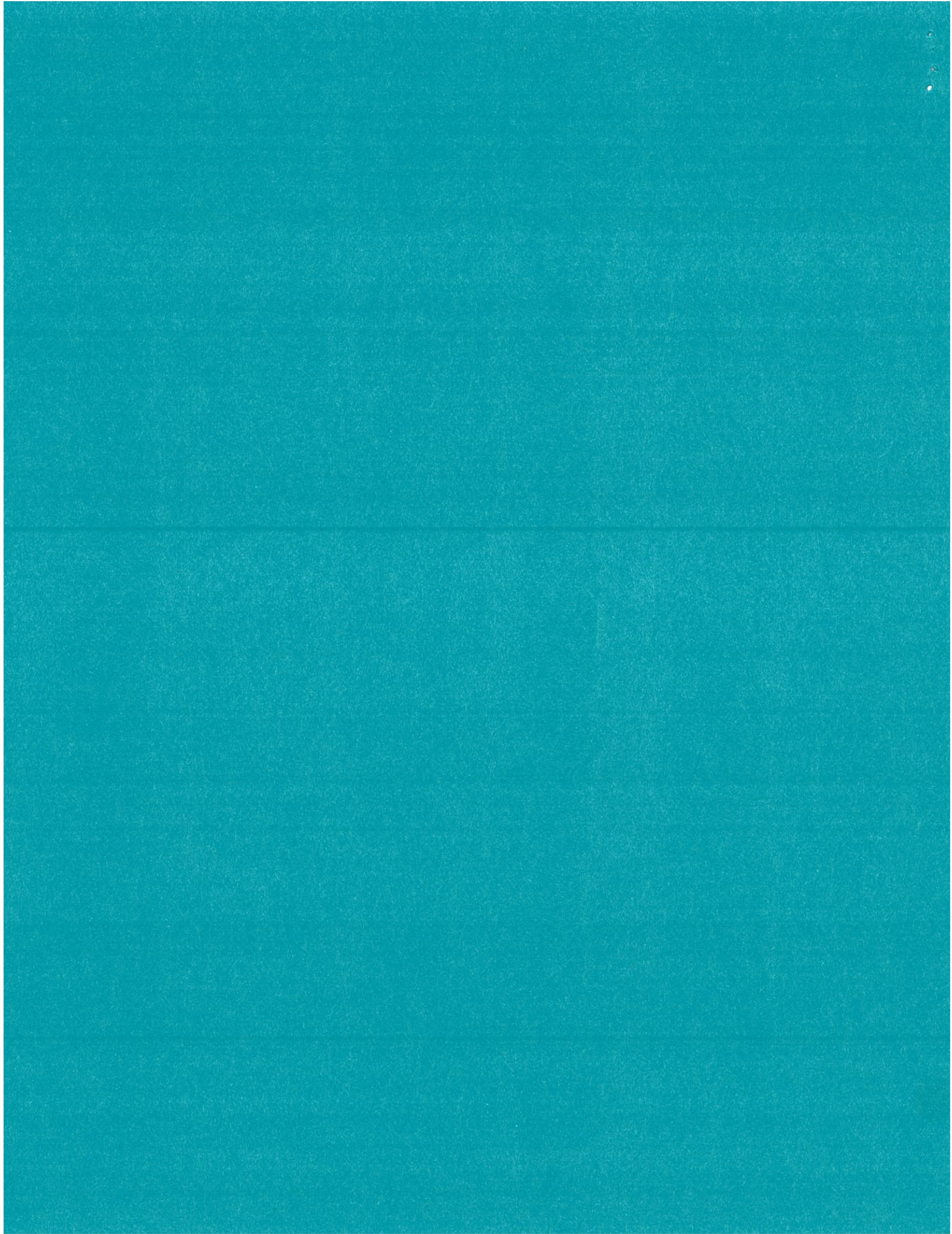


ATTACHMENT 1

Laboratory Report of Bioassay Results for

High Strength Waste Sampling

20 October 1994



**RESULTS OF BIOASSAYS CONDUCTED ON
TWO HIGH STRENGTH WASTE SAMPLES
FROM THE VAN CAMP AND STARKIST TUNA CANNERIES
IN AMERICAN SAMOA**

Prepared for:

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Ref: 9309-3

INTRODUCTION

At the request of CH2M Hill (Project # PDX 30702), Advanced Biological Testing conducted acute effluent bioassay testing on *Mysidopsis bahia*, *Mytilus edulis*, and *Citharichthys stigmaeus* using high strength wastes (HSW) collected separately from the Starkist (HSW-1) and Van Camp (HSW-2) tuna canneries in American Samoa. The study was run using methods generally specified in EPA 1991 and in a Sampling and Testing Plan submitted to the EPA.

The study was conducted at the Advanced Biological Testing Laboratory in Tiburon, California, and was managed by Mr. Mark Fisler.

2.1 EFFLUENT SAMPLING

The high strength wastes were sampled as composites on October 20, 1994 by personnel from the two canneries. Due to shipping and airline scheduling problems, frequently encountered in this region, the sample was received by the laboratory on October 24, 1994. A single gallon carboy was provided from each cannery and were labeled at ABT as HSW-1 (HSW-SKS Grab) and HSW-2 (Pipeline Sludge HS-W2, Van Camp). Samples were maintained in ice-filled coolers from the date of sampling until laboratory receipt. The samples were at 2-3°C upon receipt and were stored at 4°C until use.

2.2 SAMPLE PREPARATION AND TESTING METHODS

2.2.1 Testing on the speckled sanddab, *Citharichthys stigmaeus*

In agreement with the EPA regarding the proposed testing concentrations, the high strength wastes were tested at six concentrations starting from 2.0% and dropping using a 50% dilution factor. The final concentrations were 2.0, 1.0, 0.5, 0.25, 0.125, and 0.06% as vol:vol dilutions in seawater. The diluent was filtered seawater from San Francisco Bay. The dilutions were brought up to the test temperature ($17 \pm 2^\circ\text{C}$) and aerated continuously. These effluents have an extremely high biological oxygen demand, therefore aeration was carried out from the beginning of the test.

A reference toxicant was run using concentrations of the toxicant Sodium Dodecyl Sulfonate (SDS) made up as a 2 grams per liter stock solution in distilled water. The tested concentrations were set at 25, 12.5, 6.25, 3.1, and 1.6 mg/L in 30 ppt seawater in a 24 hour test.

The bioassays were carried out on juvenile *Citharichthys stigmaeus*, supplied by J. Brezina and Associates in Dillon Beach, California. The animals were received at ABT on October 24, 1994. The test conditions are summarized in Table 1. Five replicates of each concentration were tested with ten juvenile fish per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

2.2.2 Testing on the mysid, *Mysidopsis bahia*

In agreement with the EPA regarding the proposed testing concentrations, the high strength wastes were tested at six concentrations starting from 2.0% and dropping using a 50% dilution factor. The final concentrations were 2.0, 1.0, 0.5, 0.25, 0.125, and 0.06% as vol:vol dilutions in seawater. The diluent was filtered seawater from San Francisco Bay. The dilutions were brought up to the test temperature ($16 \pm 2^\circ\text{C}$) and aerated continuously.

A reference toxicant was run using concentrations of the toxicant Sodium Dodecyl Sulfonate (SDS) made up as a 2 grams per liter stock solution in distilled water. The tested concentrations were set at 40, 20, 10, 5, 2.5 and 1.25 mg/L in 30 ppt seawater in a 96 hour test.

The first bioassay was carried out on 7-10 day old larval *Mysidopsis bahia*, supplied by Aquatox from Hot Springs, Arkansas. The animals were received at ABT on November 1, 1994. The test conditions for this test are summarized in Table 2. Five replicates of each concentration were tested with ten larval mysids per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

2.2.3 Bivalve Larval Bioassay

Test solutions used in the bioassays were prepared using San Francisco Bay seawater at 30 ppt in serial dilution (0.5) to create 2.0, 1.0, 0.5, 0.25, 0.125, and 0.06% test concentrations for the bioassays. The bivalve study was conducted under ASTM 1993 guidelines.

The reference toxicant for the bivalve larval bioassays was copper sulfate at test concentrations of 3.75, 7.5, 15, 30, and 60 $\mu\text{g/L}$.

The bivalve larvae survival and development test was run following methods in ASTM (1993). Bay mussels, *Mytilus edulis*, were obtained from A. K. Siewers, Santa Cruz, California. Adults were induced to spawn by heat shocking. Released gametes were placed in individual containers of filtered seawater and examined for viability. Gametes were mixed and allowed to fertilize for up to two hours, under gentle aeration. Fertilized eggs were then separated from sperm and debris by filtering the suspension at 20 μm . Egg stock density was estimated by counting an aliquot of dilute stock concentrate. Equal volumes of concentrate were added to each replicate to

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an initial density of 15-30 embryos per mL. Initial stocking density was confirmed by counting a 5 mL aliquot from at least three control replicates.

Testing was conducted at $16 \pm 2^{\circ}\text{C}$ under a 14 hour light and 10 hour dark photoperiod. Temperature, pH, dissolved oxygen, and salinity were recorded at 0 and 48 hours; temperature was also recorded at 24 hours. Total ammonia in the 2% concentration was 3.6 mg/L at test initiation for HSW-1 and 6.1 mg/L for HSW-2. Ammonia was not measured on Day 2. At the end of the exposure period, a 5 mL sub-sample was taken from each test replicate and preserved with buffered formalin. Sub-samples were counted in a Sedgwick-Rafter cell, and the total number of normal and abnormal larvae were counted.

Gentle aeration was initiated on Day 0, and continued for the duration of the tests. To assess the effects of aeration, an aeration control was run simultaneously. No statistical differences were observed between aerated and unaerated controls.

2.3 STATISTICAL ANALYSIS

At the conclusion of the testing, the survival data were evaluated statistically using ToxCalc™ to determine ECp, NOEC, and LOEC values where appropriate. ToxCalc™ is a comprehensive statistical application that follows standard guidelines for acute and chronic toxicity data analysis. Data were evaluated statistically to estimate the LC50 and IC50 values for the tests using the Probit or Trimmed Spearman-Kärber Method.

3.1 Initial Effluent Quality

The two High Strength Wastes were tested for basic water quality parameters upon receipt at the laboratory. HSW-1 had a dissolved oxygen level of 0.7 mg/L; a pH of 6.53; a salinity of 23.5 ppt; and a total ammonia level of 480 mg/L. HSW-2 had a dissolved oxygen level of 0.6 mg/L; a pH of 6.39; a salinity of 14.0 ppt; and a total ammonia level of 350 mg/L.

3.1 *Citharichthys stigmaeus*

Water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at $17 \pm 2^\circ\text{C}$; pH remained relatively stable, and the salinity increased slightly as would be expected in a static test. The dissolved oxygen did drop as projected after test initiation in all of the concentration even with supplemental aeration and aeration was maintained in all chambers for the duration of the test. Ammonia was measured in all replicates from each concentration daily and was a potentially significant toxic component of the test for the highest three concentrations.

The LC50 for HSW-1 was 0.35% based upon a Trimmed Spearman-Kärber method. The majority of the observed toxicity again occurred in the first 24 hours. There was significant mortality at 2.0, 1.0, and 0.5% concentrations compared to the control at 96 hours. The NOEC was 0.25% and the LOEC was 0.5%

The LC50 for HSW-2 was 0.37% based upon a Trimmed Spearman-Kärber method. The majority of the observed toxicity occurred in the first 24 hours. There was significant mortality at 2.0, 1.0, and 0.5% concentrations compared to the control at 96 hours. The NOEC was 0.25%, and the LOEC was 0.5%.

The reference toxicant test required the use of the Trimmed Spearman-Kärber method and generated an LC50 of 3.9 mg/L, an NOEC of 3.1 mg/L, and an LOEC of 6.25 mg/L. This is the third reference toxicant test on *Citharichthys* at this laboratory, therefore no database has been established by this laboratory although the data has been consistent in the 3 - 4 mg/L range. The current laboratory mean is 3.92 mg/L.

3.2 *Mysidopsis bahia*

Water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at $17 \pm 2^{\circ}\text{C}$; pH remained relatively stable, and the salinity increased slightly as would be expected in a static test. The dissolved oxygen did drop as projected after test initiation in all of the concentration even with supplemental aeration and aeration was maintained in all chambers for the duration of the test. Ammonia was measured in all replicates from each concentration daily and was a potentially significant toxic component of the test for the highest three concentrations.

The LC50 for HSW-1 was 1.16%. At 96 hours, there was significant mortality at 2.0 and 1.0% concentrations compared to the control. The NOEC was 0.5% and the LOEC was 1.0%.

The LC50 for HSW-2 was 0.79%. again there was significant mortality at 96 hours in the 2.0 and 1.0% concentrations compared to the control. The NOEC was 0.5%, and the LOEC was 1.0%.

The reference toxicant test had an LC50 of 7.27 mg/L, with an NOEC of 1.25 mg/L and an LOEC of 2.5 mg/L. This is the third reference toxicant test on *Mysidopsis* at this laboratory, therefore no database has been established. The current mean is 13.5 mg/L.

3.3 BIVALVE LARVAL BIOASSAY

Water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at $17 \pm 2^{\circ}\text{C}$; pH remained relatively stable, and the salinity increased slightly as would be expected in a static test. The dissolved oxygen did drop as projected after test initiation in all of the concentration even with supplemental aeration and aeration was maintained in all chambers for the duration of the test. Ammonia was measured in all replicates from each concentration daily and was a potentially significant toxic component of the test for the highest three concentrations.

Control survival was acceptable at 100% with 1.4% abnormal development. The LC50 for HSW-1 was $>2.0\%$, while the LC50 for HSW-2 was 0.2%. The IC50 for HSW-1 was 0.1% and the IC50 for HSW-2 was 0.18%.

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The LC50 (6.1 $\mu\text{g/L}$) for the copper sulfate reference toxicant test was within two standard deviations of the laboratory mean of 15.9 $\mu\text{g/L}$ indicating normal to higher sensitivity of the test organisms.

3.5 AMMONIA MEASUREMENTS

Ammonia in both of the HSW was very high. When measured in a 25% dilution in seawater, ammonia levels ranged from 88 to 120 mg/L. When converted to the 100% concentration, the ammonia level would be above 350 - 450 mg/L. The un-ionized fraction as NH_3 would range from 17 to 24 mg/L at 100% concentration.

4 bay av. 0.035 mg/L 1/3 yrs
1 hr av .233 mg/L 13 yrs

TABLE 1

**Bioassay Procedure And Organism Data
For the Survival Bioassay
Using *Citharichthys stigmaeus* (U.S. EPA 1991)**

<u>Parameter</u>	<u>Data</u>
<u>Test Species</u>	<i>Citharichthys stigmaeus</i>
Supplier	J. Brezina and Associates
Collection location	Tomales Bay
Date Acquired	10/25/94
Acclimation Time	24 hours
Acclimation Water	30 ppt seawater
Acclimation Temperature	12 ± 2°C
Age group	Juveniles, 3-5 cm TL
<u>Sample Identification</u>	
Sample ID(s)	941024-19, -20
Date Sampled	10/20/94
Date Received at ABT	10/24/94
Volume Received	One gallon
Sample Storage Conditions	4°C in the dark
<u>Test Procedures</u>	
Type; Duration	96 hour static acute, renewal at 48 hours
Test Dates	10/26/94 to 10/30/94
Control Water	San Francisco Bay seawater
Test Temperature	17 ± 2°C
Test Photoperiod	16 L : 8 D
Initial Salinity	31 ± 2 ppt
Test Chamber	10 L polyethylene chamber
Animals/Replicate	10 animals/replicate
Exposure Volume	5 L
Replicates/Treatment	5
Feeding	None
Deviations from procedures	None

TABLE 2

**Bioassay Procedure And Organism Data
For the Survival Bioassay
Using *Mysidopsis bahia* (U.S. EPA 1991)**

<u>Parameter</u>	<u>Data</u>
<u>Test Species</u>	<i>Mysidopsis bahia</i>
Supplier	Aquatox, Arkansas
Date Acquired	11/1/94
Acclimation Time	None
Acclimation Water	Shipping water
Acclimation Temperature	20 ± 2°C
Age group	7-10 day larvae
<u>Sample Identification</u>	
Sample ID(s)	941024-19, -20
Date Sampled	10/20/94
Date Received at ABT	10/24/94
Volume Received	Five gallons
Sample Storage Conditions	4°C in the dark
<u>Test Procedures</u>	
Type; Duration	Acute; static; renewal at 48 hours
Test Dates	11/1/94 to 11/5/94
Control Water	San Francisco Bay seawater
Test Temperature	18 ± 2°C
Test Photoperiod	14 L : 10 D
Initial Salinity	30 ppt
Test Chamber	1000 mL jars
Animals/Replicate	10 animal/replicate
Exposure Volume	500 mL
Replicates/Treatment	5
Feeding	Brine shrimp (24 hr old nauplii)
Deviations from procedures	None

TABLE 3

**Bioassay Procedure And Organism Data
For The 48 Hour Bioassay
Using Larvae of *Mytilus edulis* (ASTM 1993)**

<u>Parameter</u>	<u>Data</u>
<u>Test Species</u>	<i>Mytilus edulis</i>
Supplier	A.K. Siewers, Santa Cruz, CA
Date Acquired	10/25//94
Acclimation Time	None
Acclimation Water	Not applicable
Acclimation Temperature	Not applicable
Age group	Fertilized embryos, 2 hours
<u>Sample Identification</u>	
Sample ID(s)	941024-19, -20
Date Sampled	10/20/94
Date Received at ABT	10/24/94
Volume Received	One gallon
Sample Storage Conditions	4°C in the dark
<u>Test Procedures</u>	
Type; Duration	Acute; static; 48 hours
Test Dates	10/25/94 to 10/27/94
Control Water	San Francisco Bay seawater
Test Temperature	16 ± 2°C
Test Photoperiod	16 L : 8 D
Salinity	32 ± 2 ppt
Test Chamber	125 mL beakers
Animals/Replicate	Approximately 30 embryos per mL
Exposure Volume	100 mL
Replicates/Treatment	3
Feeding	None
Deviations from procedures	Chambers were gently aerated with low bubble aeration

TABLE 4
SUMMARY OF RESULTS
FOR THE HIGH STRENGTH WASTE BIOASSAYS

Species	Test	Endpoint	HSW-1	HSW-2
<i>Citharichthys stigmaeus</i>	96 hr static	LC50	0.35%	0.37%
		NOEC	0.25%	0.25%
		LOEC	0.50%	0.50%
<i>Mysidopsis bahia</i>	96 hr static	LC50	1.16%	0.79%
		NOEC	0.50%	0.50%
		LOEC	1.00%	1.00%
<i>Mytilus edulis</i>	48 hr static	LC50	>2.0	0.20%
		IC50	0.10%	0.18%

Note:

HSW-1: Starkist

HSW-2: Van Camp

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TABLE 5

SUMMARY OF RESULTS FOR THE REFERENCE TOXICANT TESTS

<i>Citharichthys stigmatus</i>	SDS				
Concentration (mg/L)	% Survival	LC50 (mg/L)	NOEC (mg/L)	LOEC (mg/L)	
Control	100.0	3.9	3.1	6.25	
1.6	100.0				
3.1	83.3				
6.25	0.0*				
12.5	0.0*				
25	0.0*				

Lab LC50 = 3.92.

<i>Mysidopsis bahia</i>	SDS				
Concentration (mg/L)	% Survival	LC50 (mg/L)	NOEC (mg/L)	LOEC (mg/L)	
Control	98.0	7.27	1.25	2.5	
0.7	90.0				
1.25	90.0				
2.5	73.3*				
5	83.3*				
10	70.0*				
20	10.0*				
40	0.0*				

Lab LC50 = 13.52.

Bivalve larvae	Copper sulfate				
Concentration (µg/L)	Mean Normal Larvae/mL	% Treatment Mortality	LC50 (µg/L)	(%) Abnormal	
Initial Counts	23.5		6.1		
Control W/Air	23.5	NA		1.4	
Control WO/Air	22.9	NA		3.8	
3.75	19.0	6.4		1.8	
7.5	2.3*	88.5		51.9	
15	4.7*	76.7		100	
30	0.0*	100.0		100	
60	0.0*	100.0		100	

* Statistically significant.

REFERENCES

U.S. EPA. 1991. Methods for measuring acute toxicity of effluents to freshwater and marine organisms, 4th ed. EPA 600/4-90/027, September, 1991.

ASTM. 1993. Annual Book of Standards. Vol. 11.04. Standard guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve mollusca. E-724-89.

A
P
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E
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X

ANALYTICAL DATA

A

APPENDIX TABLE 1

SAMPLE WATER QUALITY

Sample	pH (units)	DO (mg/L)	Total NH3 (mg/L)	Initial Salinity (ppt)
HSW-1	6.53	0.7	480	23.5
HSW-2	6.39	0.6	350	14

APPENDIX TABLE 2

Mytilus edulis

WATER QUALITY MEASUREMENTS FOR THE EFFLUENT TEST

Test Dates: 10/25-10/27/94

Concentration (%) Rep		Day 0				Day 1 °C	Day 2			
		pH	DO	°C	Sal		pH	DO	°C	Sal
Control	1	8.06	8.8	16.7	32	16.2	8.00	8.8	16.9	32
W/Air	2					16.3	8.01	8.8	16.9	32
	3					16.2	8.02	8.6	16.9	32
Control	1	8.06	8.8	16.7	32	16.2	8.09	8.8	16.9	32
WO/Air	2					16.2	8.11	8.8	16.9	32
	3					16.2	8.13	8.8	16.9	32
HSW-1										
0.06	1	8.04	8.8	16.8	32	16.3	8.12	8.8	16.9	32
	2					16.2	8.09	8.7	16.9	32
	3					16.2	8.11	8.8	16.9	32
0.125	1	7.99	8.8	16.8	32	16.3	8.14	8.6	16.9	32
	2					16.2	8.08	8.6	16.9	33
	3					16.2	8.12	8.7	16.9	32
0.25	1	7.88	8.8	16.7	32	16.2	8.14	8.6	16.9	33
	2					16.2	8.12	8.6	16.9	32
	3					16.3	8.08	8.5	16.9	32
0.5	1	7.68	8.8	16.6	32	16.2	8.02	6.2	16.9	32
	2					16.2	7.75	6.0	16.9	32
	3					16.2	7.68	6.1	16.9	32
1	1	7.34	8.8	16.6	32	16.2	8.01	4.8	16.9	32
	2					16.3	8.00	4.9	16.9	32
	3					16.3	7.93	4.8	16.9	32
2	1	6.96	8.4	16.6	32	16.2	8.04	3.4	16.9	32
	2					16.2	7.99	3.2	16.9	32
	3					16.2	8.05	3.4	16.9	32
Min		6.96	8.4	16.6	32	16.2	7.68	3.2	16.9	32
Max		8.06	8.8	16.8	32	16.3	8.14	8.8	16.9	33

APPENDIX TABLE 2 (Cont'd)

Mytilus edulis

WATER QUALITY MEASUREMENTS FOR THE EFFLUENT TEST

Test Dates: 4/7-4/9/94

Concentration (%)	Rep	Day 0				Day 1 °C	Day 2				
		pH	DO	°C	Sal		pH	DO	°C	Sal	
HSW-2											
0.06	1	8.06	8.8	16.7	32	16.3	8.12	8.6	16.9	32	
	2					16.3	8.15	8.5	16.9	32	
	3					16.3	8.16	8.6	16.9	32	
0.125	1	8.04	8.9	16.6	32	16.2	8.17	8.5	16.9	32	
	2					16.2	8.17	8.5	16.8	32	
	3					16.2	8.19	8.5	16.9	32	
0.25	1	7.94	8.8	16.7	32	16.2	8.20	8.4	17.0	32	
	2					16.2	8.19	8.5	16.9	32	
	3					16.3	8.14	8.2	16.9	32	
0.5	1	7.77	8.7	16.7	32	16.3	7.73	3.4	16.9	32	
	2					16.3	8.11	7.8	16.9	32	
	3					16.3	8.15	7.8	16.9	32	
1	1	7.40	8.7	16.8	32	16.2	8.09	7.4	17.0	32	
	2					16.2	8.19	7.6	16.9	32	
	3					16.2	8.20	7.6	16.9	32	
2	1	6.92	8.6	16.6	32	16.2	8.03	3.8	16.9	32	
	2					16.2	8.03	4.8	16.9	32	
	3					16.2	7.98	4.6	16.9	32	
	Min	6.92	8.6	16.6	32	16.2	7.73	3.4	16.8	32	
	Max	8.06	8.9	16.8	32	16.3	8.20	8.6	17.0	32	

APPENDIX TABLE 3

Mytilus edulis
SUMMARY OF RESULTS FOR BIVALVE LARVAE HIGH STRENGTH WASTE BIOASSAY
Test Dates: 10/25-10/27/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
Initial Counts	1	110		22.0			
	2	135		27.0			
	3	108		21.6			
	Mean			23.5			
Final Control W/Air	1	101	0	20.2		0.0	
	2	129	0	25.8		0.0	
	3	117	5	24.4		4.1	
	Mean			23.5	100.0	1.4	NA
Final Control WO/Air	1	104	5	21.8		4.6	
	2	109	3	22.4		2.7	
	3	118	5	24.6		4.1	
	Mean			22.9	100.0	3.8	NA
HSW-1 0.06	1	82	12	18.8		12.8	
	2	89	14	20.6		13.6	
	3	78	15	18.6		16.1	
	Mean			19.3	93.4	14.2	4.8
0.125	1	23	72	19.0		75.8	
	2	18	58	15.2		76.3	
	3	20	71	18.2		78.0	
	Mean			17.5	84.4	76.7	14.0
0.25	1	3	82	17.0		96.5	
	2	1	77	15.6		98.7	
	3	3	85	17.6		96.6	
	Mean			16.7	80.8	97.3	17.6
0.5	1	0	85	17.0		100.0	
	2	0	93	18.6		100.0	
	3	0	81	16.2		100.0	
	Mean			17.3	83.4	100.0	14.9
1	1	0	89	17.8		100.0	
	2	0	94	18.8		100.0	
	3	0	97	19.4		100.0	
	Mean			18.7	90.2	100.0	8.0
2	1	0	95	19.0		100.0	
	2	0	96	19.2		100.0	
	3	0	87	17.4		100.0	
	Mean			18.5	89.5		8.7

APPENDIX TABLE 3 (Cont'd)

Mytilus edulis

SUMMARY OF RESULTS FOR BIVALVE LARVAE HIGH STRENGTH WASTE BIOASSAY

Test Dates: 10/25-10/27/94

Concentration (%)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
HSW-2 0.06	1	102	3	21.0		2.9	
	2	87	2	17.8		2.2	
	3	117	3	24.0		2.5	
	Mean			20.9	100.0	2.5	0.0
0.125	1	67	13	16.0		16.3	
	2	61	12	14.6		16.4	
	3	52	12	12.8		18.8	
	Mean			14.5	69.9	17.1	28.7
0.25	1	0	38	7.6		100.0	
	2	0	27	5.4		100.0	
	3	0	33	6.6		100.0	
	Mean			6.5	31.6	100.0	67.8
0.5	1	0	27	5.4		100.0	
	2	0	27	5.4		100.0	
	3	0	27	5.4		100.0	
	Mean			5.4	26.1	100.0	73.4
1	1	0	36	7.2		100.0	
	2	0	39	7.8		100.0	
	3	0	31	6.2		100.0	
	Mean			7.1	34.1	100.0	65.2
2	1	0	37	7.4		100.0	
	2	0	31	6.2		100.0	
	3	0	36	7.2		100.0	
	Mean			6.9	33.5	100.0	65.8

APPENDIX TABLE 4

Mytilus edulis
WATER QUALITY MEASUREMENTS
FOR THE REFERENCE TOXICANT (COPPER) TEST

Concentration µg/L	Rep	Day 0				Day 1 °C	Day 2			
		pH	DO	°C	Sal		pH	DO	°C	Sal
3.75	1	8.08	8.8	16.7	32	16.4	8.15	8.4	17.0	32
	2					16.4	8.13	8.5	16.9	32
	3					16.4	8.15	8.6	16.9	32
7.5	1	8.09	8.8	16.7	32	16.5	8.18	8.6	16.9	32
	2					16.4	8.18	8.4	16.9	32
	3					16.5	8.16	8.4	16.9	32
15	1	8.10	8.7	16.7	32	16.5	8.17	8.5	16.9	32
	2					16.5	8.18	8.5	17.0	32
	3					16.5	8.18	8.4	17.0	32
30	1	8.10	8.7	16.8	31	16.5	8.17	8.4	16.9	32
	2					16.5	8.17	8.4	16.9	32
	3					16.5	8.16	8.5	16.9	32
60	1	8.11	8.7	16.7	30	16.5	8.16	8.5	16.9	32
	2					16.4	8.17	8.6	16.9	32
	3					16.5	8.16	8.6	17.0	32
Min		8.08	8.7	16.7	30	16.4	8.13	8.4	16.9	32
Max		8.11	8.8	16.8	32	16.5	8.18	8.6	17.0	32

APPENDIX TABLE 5

Mytilus edulis
SUMMARY OF RESULTS FOR THE BIVALVE LARVAE
REFERENCE TOXICANT (COPPER) BIOASSAY

Concentration (µg/L)	Rep	Total Normal	Total Abnormal	Total Larvae/mL	% Survival	% Abnormal	Treatment Mortality (%)
3.75	1	90	2	18.4		2.2	
	2	97	1	19.6		1.0	
	3	93	2	19.0		2.1	
	Mean			19.0	91.8	1.8	6.4
7.5	1	4	5	1.8		55.6	
	2	6	7	2.6		53.8	
	3	7	6	2.6		46.2	
	Mean			2.3	11.3	51.9	88.5
15	1	0	27	5.4		100.0	
	2	0	21	4.2		100.0	
	3	0	23	4.6		100.0	
	Mean			4.7	22.9	100.0	76.7
30	1	0	0	0.0		100.0	
	2	0	0	0.0		100.0	
	3	0	0	0.0		100.0	
	Mean			0.0	0.0	100.0	100.0
60	1	0	0	0.0		100.0	
	2	0	0	0.0		100.0	
	3	0	0	0.0		100.0	
	Mean			0.0	0.0	100.0	100.0

APPENDIX TABLE 6

Mysidopsis bahia
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-1

Concentration (%) Rep		Day 0					Day 1					Day 2					Day 3					Day 4				
		pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	7.98	7.9	0.03	17.1	32	8.18	8.2		17.2	33	8.16	7.2	0.02	17.1	33	8.17	7.3	0.03	17.4	33	8.05	8.0	0.03	17.9	34
	2						8.23	8.1	0.03	17.0	33	8.23	7.2		16.5	33	8.22	7.2		17.1	33	8.14	8.0		17.7	34
	3						8.22	8.1		16.9	32	8.24	7.2		16.3	33	8.24	7.3		16.9	33	8.17	8.0		17.6	34
	4						8.22	8.4		16.6	33	8.24	7.2		16.2	33	8.24	7.4		16.8	33	8.18	8.1		17.5	34
	5						8.22	8.5		16.5	33	8.24	7.4		16.0	33	8.25	7.4		16.6	33	8.20	8.2		17.2	34
0.06	1	7.93	8.0	0.14	17.3	32	8.17	8.5		17.2	33	8.24	7.6	0.11	16.6	33	8.23	7.6	0.11	17.2	34	8.18	8.2	0.10	17.7	34
	2						8.15	8.5	0.10	17.0	32	8.25	7.5		16.5	33	8.20	7.4		17.0	33	8.13	8.2		17.6	34
	3						8.13	8.3		16.8	32	8.23	7.4		16.4	33	8.20	7.4		16.9	33	8.14	8.1		17.6	34
	4						8.20	8.2		16.5	33	8.19	7.4		16.2	33	8.13	7.4		16.6	34	7.98	8.0		17.3	34
	5						8.21	8.2		16.4	31	8.21	7.4		16.0	33	8.16	7.4		16.5	34	8.09	7.8		17.0	34
0.125	1	7.87	8.0	0.27	17.2	32	8.09	8.4		17.2	33	8.22	7.6	0.19	16.6	33	8.21	7.5	0.21	17.2	34	8.15	8.0	0.20	17.6	34
	2						8.02	8.4	0.22	17.0	33	8.24	7.5		16.5	33	8.21	7.4		17.1	33	8.16	8.0		17.6	34
	3						8.01	8.5		16.8	32	8.21	7.4		16.2	33	8.21	7.4		16.8	33	8.14	8.0		17.4	34
	4						8.03	8.3		16.5	33	8.25	7.4		16.0	33	8.25	7.4		16.5	34	8.21	8.0		17.0	34
	5						8.14	8.4		15.9	33	8.25	7.4		16.0	33	8.26	7.4		16.5	34	8.22	8.0		16.9	34
0.25	1	7.72	8.1	0.51	17.2	32	8.01	8.2		17.2	33	8.27	7.6	0.38	16.7	33	8.26	7.6	0.40	17.1	34	8.21	8.2	0.39	17.5	34
	2						8.01	8.2	0.70	17.0	33	8.26	7.6		16.5	33	8.27	7.6		17.0	34	8.20	8.0		17.5	34
	3						7.85	7.7		16.9	32	8.17	7.4		16.4	33	8.21	7.5		16.9	33	8.12	8.0		17.4	34
	4						8.02	7.8		16.5	33	8.23	7.4		16.0	33	8.22	7.4		16.6	34	8.15	7.8		17.0	34
	5						8.09	8.6		16.0	33	8.24	7.4		16.0	33	8.25	7.4		16.4	34	8.19	7.8		16.9	34
0.5	1	7.55	8.1	0.93	17.2	32	7.97	6.6		17.2	33	8.10	7.6	0.70	16.6	33	8.28	7.6	0.60	17.2	33	8.27	8.0	0.74	17.6	34
	2						7.84	7.7	0.40	17.0	32	8.20	7.4		16.5	33	8.23	7.5		17.0	33	8.19	8.0		17.6	34
	3						7.73	6.8		16.9	32	8.16	7.3		16.5	33	8.21	7.4		16.9	33	8.24	7.9		17.4	34
	4						7.78	7.6		16.6	33	8.13	7.2		16.3	33	8.21	7.4		16.6	34	8.18	7.8		17.2	34
	5						7.77	7.9		16.2	33	8.13	7.2		16.0	33	8.20	7.4		16.5	34	8.13	7.8		16.9	34
1	1	7.18	7.8	1.80	17.2	32	7.66	6.9		17.2	32	8.18	7.4	1.44	16.9	33	8.23	7.6	1.26	17.2	33	8.20	7.8	1.18	17.7	34
	2						7.81	7.1	1.50	17.0	32	8.23	7.3		16.6	33	8.28	7.4		17.1	33	8.26	7.8		17.7	34
	3						7.65	6.3		17.0	32	8.18	7.2		16.5	33	8.27	7.4		17.1	33	8.12	7.6		17.6	34
	4						7.60	5.9		16.7	33	8.14	7.2		16.2	33	8.23	7.3		16.7	32	8.17	7.6		17.3	34
	5						7.51	5.2		16.5	33	8.07	7.2		16.0	33	8.16	7.3		16.3	34	8.14	7.4		17.0	34
2.0	1	6.84	7.7	3.60	17.2	32	7.56	3.5		15.9	33	8.22	7.2	2.82	16.0	33	8.30	7.3	2.16	16.3	34	8.31	7.4	2.07	16.8	34
	2						7.47	2.0	3.70	15.7	33	8.09	7.2		16.0	34	—	—	—	—	—	—	—	—	—	—
	3						7.49	2.0		15.6	33	8.05	6.7		16.0	34	—	—	—	—	—	—	—	—	—	
	4						7.38	0.6		15.8	33	8.14	6.7		16.0	34	—	—	—	—	—	—	—	—	—	
	5						7.66	3.8		15.9	34	8.18	6.9		16.0	34	8.30	7.4		16.2	34	8.31	7.6		16.7	34
Min		6.84	7.7	0.03	17.1	32	7.38	0.6	0.03	15.6	31	8.05	6.7	0.02	16.0	33	8.13	7.2	0.03	16.2	32	7.98	7.4	0.03	16.7	34
Max		7.98	8.1	3.60	17.3	32	8.23	8.6	3.70	17.2	34	8.27	7.6	2.82	17.1	34	8.30	7.6	2.16	17.4	34	8.31	8.2	2.07	17.9	34

Note: — = All animals dead.

APPENDIX TABLE 6 (Cont'd)

Mysidopsis bahia
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
HSW-2

Concentration (%)	Rep	Day 0					Day 1					Day 2					Day 3					Day 4				
		pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
0.06	1	7.84	8.1	0.24	17.6	32	8.15	8.1		17.2	33	8.26	7.2	0.16	16.6	33	8.28	7.6	0.20	17.1	34	8.27	8.2	0.18	17.6	34
	2						8.02	8.0	0.28	16.9	33	8.19	7.2		16.4	33	8.20	7.5		16.9	34	8.18	8.1		17.4	34
	3						8.18	8.0		16.5	33	8.24	7.2		16.0	33	8.26	7.4		16.7	34	8.24	8.1		17.2	34
	4						8.20	8.1		16.3	33	8.26	7.4		16.0	33	8.26	7.4		16.5	34	8.26	8.0		17.0	34
	5						8.20	8.0		16.2	34	8.25	7.4		16.0	33	8.15	7.5		16.5	34	8.27	8.0		17.0	34
0.125	1	7.79	8.1	0.47	17.7	32	8.12	8.1		17.2	33	8.25	7.5	0.27	16.5	34	8.28	7.4	0.32	17.0	34	8.27	8.2	0.28	17.4	34
	2						8.11	8.0	0.32	16.9	33	8.25	7.4		16.4	33	8.27	7.4		16.8	34	8.26	8.2		17.4	34
	3						8.05	8.0		16.6	33	8.21	7.4		16.2	33	8.26	7.4		16.6	34	8.12	8.0		17.2	34
	4						8.15	8.0		16.2	33	8.23	7.3		16.1	33	8.26	7.4		16.5	34	8.21	7.6		17.0	34
	5						8.17	8.1		16.2	33	8.27	7.4		16.0	34	8.27	7.6		16.5	34	8.26	7.6		16.9	34
0.25	1	7.66	8.0	0.84	17.6	32	7.95	7.8		17.1	33	8.24	7.4	0.54	16.4	33	8.26	7.6	0.51	16.9	34	8.25	8.0	0.47	17.4	34
	2						7.89	7.8	0.60	16.9	33	8.18	7.4		16.3	33	8.24	7.4		16.9	34	8.20	8.0		17.4	34
	3						7.93	7.8		16.6	33	8.20	7.2		16.2	33	8.24	7.4		16.6	34	8.21	7.9		17.2	34
	4						7.92	7.8		16.5	33	8.20	7.2		16.1	33	8.22	7.4		16.5	34	8.19	7.8		17.0	34
	5						8.01	7.8		16.2	33	8.20	7.2		16.0	34	8.25	7.4		16.5	34	8.23	7.8		16.9	34
0.5	1	7.43	7.9	1.60	17.6	32	7.89	7.8		17.1	33	8.25	7.4	1.10	16.2	33	8.27	7.5	1.05	16.8	34	8.26	8.0	0.98	17.2	34
	2						7.83	7.8	1.21	16.9	33	8.21	7.4		16.2	33	8.27	7.4		16.7	34	8.27	7.9		17.2	34
	3						7.79	7.4		16.7	33	8.20	7.2		16.1	33	8.27	7.4		16.6	34	8.23	7.8		17.2	34
	4						7.77	7.4		16.5	33	8.16	7.2		16.0	33	8.25	7.4		16.5	34	8.21	7.6		17.0	34
	5						7.94	7.8		16.2	33	8.24	7.2		16.0	34	8.30	7.4		16.5	34	8.28	7.6		16.9	34
1	1	7.10	7.8	3.20	17.6	32	7.64	5.8		16.9	33	8.25	7.3	2.21	16.0	34	—	—	—	—	—	—	—	—	—	—
	2						7.50	0.8	2.57	16.9	33	8.15	7.3		16.0	33	—	—	—	—	—	—	—	—	—	—
	3						7.62	5.2		16.6	33	8.20	7.2		16.0	33	8.24	7.4	2.05	16.5	34	8.28	7.8	2.01	17.0	34
	4						7.62	5.0		16.4	33	8.21	7.2		16.1	33	8.29	7.4		16.5	34	8.31	7.6		16.9	34
	5						7.67	4.8		16.2	33	8.17	7.2		16.0	34	8.25	7.3		16.5	34	8.22	7.6		16.9	34
2.0	1	6.82	7.2	6.10	17.9	32	7.45	0.8		17.0	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.49	0.4	5.28	16.7	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.40	0.6		16.5	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	4						7.57	1.8		16.3	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	5						7.47	0.6		16.2	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Mln		6.82	7.2	0.24	17.6	32	7.40	0.4	0.28	16.2	33	8.15	7.2	0.16	16.0	33	8.15	7.3	0.20	16.5	34	8.12	7.6	0.18	16.9	34
Max		7.84	8.1	6.10	17.9	32	8.20	8.1	5.28	17.2	34	8.27	7.5	2.21	16.6	34	8.30	7.6	2.05	17.1	34	8.31	8.2	2.01	17.6	34

Note: — = All animals dead.

APPENDIX TABLE 7

Mysidopsis bahia
SURVIVAL DATA FOR EFFLUENT TEST
HSW-1

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	98.0
	2	10	10	10	10	10	100	
	3	10	10	9	9	9	90	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.06	1	10	10	9	9	9	90	90.0
	2	10	10	9	10	10	100	
	3	10	10	10	9	9	90	
	4	10	9	9	8	8	80	
	5	10	9	9	9	9	90	
0.125	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.25	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.5	1	10	10	10	10	10	100	98.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	9	9	90	
1	1	10	10	10	10	9	90	66.0
	2	10	10	10	10	6	60	
	3	10	10	10	10	7	70	
	4	10	10	10	10	6	60	
	5	10	10	8	6	5	50	
2	1	10	*	3	3	1	10	4.0
	2	10	*	0	—	—	0	
	3	10	*	0	—	—	0	
	4	10	*	0	—	—	0	
	5	10	*	2	2	1	10	

Notes: — = All animals dead.

* Sample too turbid to do counts.

APPENDIX TABLE 7 (Cont'd)

Mysidopsis bahia
SURVIVAL DATA FOR EFFLUENT TEST
HSW-2

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
0.06	1	10	10	10	10	10	100	80.0
	2	10	10	7	6	5	50	
	3	10	10	10	10	10	100	
	4	10	10	7	7	6	60	
	5	10	10	9	9	9	90	
0.125	1	10	10	10	10	10	100	94.0
	2	10	10	9	9	8	80	
	3	10	10	10	10	9	90	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.25	1	10	10	10	10	9	90	86.0
	2	10	10	10	10	9	90	
	3	10	10	10	9	9	90	
	4	10	10	10	9	9	90	
	5	10	10	8	8	7	70	
0.5	1	10	10	9	9	9	90	88.0
	2	10	10	10	9	9	90	
	3	10	10	10	9	9	90	
	4	10	10	10	10	9	90	
	5	10	10	9	9	8	80	
1	1	10	*	0	—	—	0	14.0
	2	10	*	0	—	—	0	
	3	10	*	2	2	3	30	
	4	10	*	2	2	2	20	
	5	10	*	2	2	2	20	
2	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	

Notes: — = All animals dead.

* Sample too turbid to do counts.

APPENDIX TABLE 8

Mysidopsis bahia
WATER QUALITY MEASUREMENTS
FOR REFERENCE TOXICANT (S.D.S) TEST

Concentration (mg/L) Rep		Day 0			Day 1			Day 2			Day 3			Day 4							
		pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal	pH	DO	°C	Sal				
0.7	1	8.06	8.2	15.9	33	8.16	7.2	17.4	33	8.16	7.2	17.4	33	8.03	7.4	17.6	33	7.88	6.8	18.2	33
	2					8.19	7.1	17.2	33	8.16	7.2	17.3	33	8.07	7.4	17.6	33	7.91	6.7	18.2	33
	3					8.20	7.1	17.3	33	8.16	7.1	17.3	33	8.06	7.2	17.6	33	7.88	6.6	18.2	33
1.25	1	8.07	8.1	15.9	32	8.19	7.0	17.2	33	8.17	7.0	17.3	33	8.08	7.2	17.6	33	7.93	6.5	18.2	33
	2					8.19	7.0	17.0	33	8.16	7.0	17.2	33	8.07	7.2	17.6	33	7.93	6.6	18.0	33
	3					8.19	7.0	17.1	33	8.15	7.1	17.2	33	8.07	7.2	17.5	33	7.93	6.6	18.0	33
2.5	1	8.07	8.1	15.8	32	8.16	6.9	17.2	33	8.13	7.0	17.3	33	8.05	7.2	17.6	33	7.93	6.7	18.2	33
	2					8.15	6.5	17.0	33	8.12	7.0	17.0	33	8.05	7.2	17.5	33	7.96	6.6	18.0	33
	3					8.14	6.4	17.0	33	8.12	7.0	17.1	33	8.03	7.2	17.6	33	7.89	6.7	18.0	33
5	1	8.08	8.1	15.9	32	8.11	6.4	17.2	33	8.08	7.0	17.4	33	8.02	7.2	17.6	33	7.90	6.5	18.3	33
	2					8.11	6.0	17.0	33	8.08	6.8	17.3	33	8.01	7.0	17.6	33	7.91	6.5	18.1	33
	3					8.10	5.8	17.0	33	8.09	6.8	17.2	33	8.00	7.0	17.6	33	7.89	6.4	18.2	33
10	1	8.08	8.0	15.8	32	8.05	5.8	17.3	33	8.01	6.4	17.5	33	7.98	7.0	17.9	33	7.89	6.4	18.6	33
	2					8.07	5.8	17.1	33	7.99	6.4	17.3	33	7.98	7.0	17.8	33	7.89	6.4	18.3	33
	3					8.08	5.1	17.2	33	7.98	6.4	17.3	33	7.98	7.0	17.6	33	7.87	6.4	18.3	33
20	1	8.09	8.0	15.8	32	8.05	4.8	17.5	33	7.80	4.5	17.7	33	—	—	—	—	—	—	—	—
	2					8.06	4.7	17.3	33	7.77	4.4	17.6	33	7.83	7.1	18.0	33	7.85	6.4	18.7	33
	3					8.05	4.7	17.2	33	7.78	4.4	17.4	33	7.81	6.4	17.8	33	7.92	6.7	18.6	34
40	1	8.09	8.1	15.7	32	8.12	6.0	17.8	33	—	—	—	—	—	—	—	—	—	—	—	—
	2					8.17	6.2	17.8	33	—	—	—	—	—	—	—	—	—	—	—	—
	3					8.17	6.2	17.8	33	—	—	—	—	—	—	—	—	—	—	—	—
Min		8.06	8.0	15.7	32	8.05	4.7	17.0	33.0	7.77	4.4	17.0	33.0	7.81	6.4	17.5	33.0	7.85	6.4	18.0	33.0
Max		8.09	8.2	15.9	33	8.20	7.2	17.8	33.0	8.17	7.2	17.7	33.0	8.08	7.4	18.0	33.0	7.96	6.8	18.7	34.0

Note: — = All animals dead.

APPENDIX TABLE 9

Mysidopsis bahia
SURVIVAL DATA FOR REFERENCE TOXICANT (S.D.S.) TEST

Concentration (mg/L)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
0.7	1	10	10	9	8	8	80	90.0
	2	10	10	10	10	10	100	
	3	10	10	9	9	9	90	
1.25	1	10	10	9	9	9	90	90.0
	2	10	10	9	9	9	90	
	3	10	10	10	10	9	90	
2.5	1	10	10	8	8	8	80	73.3
	2	10	10	7	7	7	70	
	3	10	9	8	8	7	70	
5	1	10	10	10	10	10	100	83.3
	2	10	10	7	7	6	60	
	3	10	9	9	9	9	90	
10	1	10	10	9	8	8	80	70.0
	2	10	8	7	7	7	70	
	3	10	8	7	6	6	60	
20	1	10	2	0	—	—	0	10.0
	2	10	2	2	2	2	20	
	3	10	1	1	1	1	10	
40	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	

Note: — = All animals dead.

APPENDIX TABLE 10

Citharichthys stigmatus
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
 Study Dates: 10/26-10/30/94
 HSW-1

Concentration (%) Rep		Day 0					Day 1					Day 2					Day 3					Day 4				
		pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
Control	1	8.02	8.6	<0.01	16.5	32	8.05	8.2	0.08	16.8	32	8.03	8.8	0.08	14.6	33	7.94	6.8	0.08	15.4	33	7.95	8.2	0.09	15.7	33
	2						7.92	8.3	0.08	16.9	32	7.82	8.8	0.09	14.7	33	7.78	7.0	0.09	15.5	33	7.81	8.2	0.14	15.7	33
	3						7.91	7.8	0.07	16.9	32	7.84	9.0	0.09	14.6	33	7.79	6.8	0.07	15.5	33	7.81	7.2	0.19	15.7	33
	4						8.04	8.1	0.07	16.8	32	7.99	8.7	0.08	14.5	33	8.00	6.6	0.07	15.4	33	7.99	8.1	0.18	15.6	33
	5						8.00	8.2	0.07	16.8	32	7.99	8.8	0.09	14.6	33	7.94	6.6	0.08	15.4	33	7.97	8.1	0.17	15.6	33
0.06	1	7.95	8.6	0.16	16.4	32	7.90	8.1	0.14	16.7	32	8.00	9.0	0.17	14.6	33	7.99	7.2	0.16	15.4	33	8.00	8.1	0.29	15.7	33
	2						7.89	8.0	0.14	16.6	32	8.01	9.0	0.17	14.5	33	8.00	7.2	0.18	15.5	33	8.03	8.1	0.26	15.6	34
	3						7.95	8.0	0.14	16.5	32	8.04	9.0	0.17	14.5	33	8.04	7.0	0.14	15.4	33	8.06	8.3	0.29	15.5	34
	4						7.83	7.6	0.15	16.3	32	8.02	9.0	0.18	14.2	33	7.94	7.2	0.18	15.3	33	7.95	8.2	0.30	15.2	34
	5						7.82	7.8	0.15	16.2	32	7.97	8.9	0.18	14.2	33	7.93	7.2	0.17	15.4	33	7.96	7.9	0.31	15.0	33
0.125	1	7.93	8.6	0.23	16.4	32	7.61	5.1	0.21	16.3	32	7.99	8.9	0.21	14.2	33	7.98	7.4	0.20	15.4	33	8.01	8.1	0.35	15.3	34
	2						7.59	5.0	0.22	16.2	32	7.99	9.0	0.24	14.2	33	7.95	7.2	0.24	15.2	33	8.01	8.1	0.40	15.2	34
	3						7.76	7.2	0.22	16.0	32	8.01	9.1	0.23	14.2	33	7.97	7.2	0.20	15.4	33	8.03	8.2	0.48	15.4	34
	4						7.64	5.6	0.19	16.2	32	8.01	9.1	0.23	14.3	33	7.97	7.0	0.19	15.2	33	8.00	8.1	0.53	15.3	34
	5						7.86	7.3	0.19	16.2	32	8.03	9.1	0.23	14.2	33	8.04	7.0	0.21	15.3	33	8.08	8.0	0.51	15.2	34
0.25	1	7.83	8.6	0.47	16.5	32	7.58	4.6	0.35	16.0	32	7.94	9.0	0.37	13.9	34	7.90	7.2	0.34	15.3	33	7.97	8.1	0.53	14.5	36
	2						7.65	4.7	0.36	16.0	32	8.04	8.8	0.37	14.0	33	8.01	7.3	0.33	15.3	33	8.10	8.0	0.62	14.7	35
	3						7.62	4.6	0.35	16.0	32	8.07	8.9	0.36	14.3	33	8.03	7.3	0.37	15.4	33	8.10	8.2	0.57	14.9	34
	4						7.67	4.7	0.34	15.9	32	8.03	9.0	0.36	14.4	33	7.92	7.3	0.36	15.4	33	8.03	8.2	0.66	15.1	34
	5						7.67	4.8	0.34	16.0	32	8.08	9.1	0.36	14.3	33	8.05	7.2	0.37	15.3	33	8.11	8.3	0.61	14.9	35
0.5	1	7.63	8.5	0.92	16.4	32	7.50	1.2	0.74	16.5	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.50	0.9	0.67	16.6	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.52	0.8	0.76	16.6	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.51	1.3	0.75	16.6	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.57	1.0	0.66	16.6	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1	1	7.33	8.5	1.98	16.4	31	7.45	0.8	1.58	16.5	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.46	0.9	1.62	16.5	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.47	0.6	1.59	16.5	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.48	0.8	1.54	16.4	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.46	0.8	1.63	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	1	6.99	8.1	3.95	16.5	31	7.41	0.6	3.18	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2						7.40	0.4	3.20	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.48	0.6	3.12	16.0	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4						7.41	0.8	3.15	16.1	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5						7.45	0.8	3.19	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Min		6.99	8.1	<0.10	16.4	31	7.40	0.4	0.07	15.9	32	7.82	8.7	0.08	13.9	33	7.78	6.6	<0.10	15.2	33	7.81	7.2	0.09	14.5	33
Max		8.02	8.6	3.95	16.5	32	8.05	8.3	3.20	16.9	32	8.08	9.1	0.37	14.7	34	8.05	7.4	0.37	15.5	33	8.11	8.3	0.66	15.7	36

Note: — = All animals dead.

APPENDIX TABLE 10 (Cont'd)

Citharichthys stigmaeus
WATER QUALITY MEASUREMENTS FOR EFFLUENT TEST
Study Dates: 10/26-10/30/94
HSW-2

Concentration (%)	Rep	Day 0					Day 1					Day 2					Day 3					Day 4				
		pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal	pH	DO	NH3	°C	Sal
0.06	1	8.00	8.5	0.19	16.5	32	7.76	7.0	0.20	16.5	32	8.03	9.2	0.17	14.8	32	8.07	7.4	0.17	15.5	33	8.09	8.2	0.17	15.5	33
	2						7.84	7.2	0.17	16.4	32	8.03	9.1	0.17	14.4	33	8.04	7.2	0.16	15.4	33	8.08	8.3	0.20	15.5	33
	3						7.84	7.2	0.18	16.3	32	8.02	9.1	0.18	14.2	33	8.05	7.2	0.18	15.5	33	8.08	8.3	0.21	15.3	34
	4						7.75	6.2	0.17	16.4	32	8.00	9.0	0.18	14.2	33	8.01	7.0	0.17	15.5	33	8.06	8.2	0.19	15.2	34
	5						7.79	6.6	0.18	15.9	32	8.04	8.9	0.18	14.5	33	8.05	7.1	0.19	15.4	33	8.10	8.2	0.23	14.4	36
0.125	1	7.94	8.6	0.30	16.5	32	7.70	6.4	0.27	16.2	32	7.99	8.9	0.26	14.2	33	8.02	7.5	0.21	15.4	33	8.06	8.3	0.31	15.3	34
	2						7.81	6.2	0.27	16.3	32	8.03	9.1	0.27	14.3	33	8.04	7.3	0.25	15.4	33	8.09	8.1	0.34	15.3	34
	3						7.81	6.0	0.27	16.4	32	8.04	9.2	0.26	14.3	33	8.05	7.2	0.25	15.5	33	8.10	8.3	0.29	15.3	34
	4						7.58	6.1	0.29	15.9	32	8.04	9.2	0.26	13.8	33	8.06	7.2	0.27	15.3	33	8.11	8.3	0.31	14.8	35
	5						7.76	6.2	0.29	15.9	32	8.06	9.2	0.25	13.8	33	8.07	7.2	0.27	15.3	33	8.13	8.3	0.34	14.8	34
0.25	1	7.79	8.6	0.62	16.4	32	7.70	4.2	0.57	15.9	32	7.94	9.2	0.47	13.9	33	8.00	7.4	0.44	15.2	33	8.05	8.3	0.47	14.9	34
	2						7.70	4.5	0.58	15.9	32	7.91	8.9	0.47	13.8	33	7.96	7.2	0.41	15.3	33	8.02	8.2	0.49	14.9	34
	3						7.64	4.6	0.55	15.9	32	7.98	8.8	0.47	13.8	33	7.99	7.2	0.41	15.3	33	8.07	8.0	0.41	14.8	34
	4						7.61	4.6	0.53	16.1	32	7.89	8.8	0.46	14.0	33	7.92	7.3	0.40	15.3	33	8.00	8.1	0.47	15.2	34
	5						7.59	4.6	0.52	16.2	32	7.92	8.8	0.47	14.2	33	7.91	7.2	0.43	15.3	33	7.98	7.9	0.49	15.2	34
0.5	1	7.54	8.7	1.24	16.5	32	7.57	1.6	1.07	16.2	32	7.97	8.7	0.87	14.0	33	8.04	7.0	0.79	15.4	33	8.08	8.2	0.74	14.9	34
	2						7.49	1.8	1.16	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3						7.54	1.8	1.09	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	4						7.56	1.8	1.08	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	5						7.57	1.9	1.03	16.3	32	8.05	8.8	0.86	14.2	33	8.09	7.0	0.83	15.4	33	8.15	8.2	0.69	15.0	35
1	1	7.23	8.6	2.41	16.5	32	7.61	0.9	2.10	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	2						7.62	0.9	2.24	16.3	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	3						7.54	1.0	2.22	16.4	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	4						7.54	0.8	2.31	15.8	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	5						7.51	0.8	2.31	15.7	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
2.0	1	6.86	8.3	5.15	16.5	31	7.80	0.6	4.88	15.8	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	2						7.56	0.6	4.47	15.9	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	3						7.60	0.8	4.65	15.9	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	4						7.60	0.8	4.40	16.0	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	5						7.56	0.6	4.32	16.2	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Mln		6.86	8.3	0.19	16.4	31	7.49	0.6	0.17	15.7	32	7.89	8.7	0.17	13.8	32	7.91	7.0	<0.10	15.2	33	7.98	7.9	0.19	14.4	33
Max		8.00	8.7	5.15	16.5	32	7.84	7.2	4.88	16.5	32	8.06	9.2	0.87	14.8	33	8.09	7.5	0.83	15.5	33	8.15	8.3	0.74	15.5	36

Note: — = All animals dead.

APPENDIX TABLE 11

Citharichthys stigmaeus
SURVIVAL DATA FOR EFFLUENT TEST
HSW-1

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
Control	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.06	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.125	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.25	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.5	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	
1	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	
2	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	

Note: — = All animals dead.

APPENDIX TABLE 11 (Cont'd)

Citharichthys stigmaeus
SURVIVAL DATA FOR EFFLUENT TEST
HSW-2

Concentration (%)	Rep	Initial Added	Day 1	Day 2	Day 3	Day 4	% Survival	Average % Survival
0.06	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.125	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	9	10	10	100	
0.25	1	10	10	10	10	10	100	100.0
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
	5	10	10	10	10	10	100	
0.5	1	10	4	2	2	2	20	8.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	2	2	2	2	20	
1	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	
2	1	10	0	—	—	—	0	0.0
	2	10	0	—	—	—	0	
	3	10	0	—	—	—	0	
	4	10	0	—	—	—	0	
	5	10	0	—	—	—	0	

Note: — = All animals dead.

APPENDIX TABLE 12

Citharichthys stigmaeus WATER QUALITY MEASUREMENTS FOR REFERENCE TOXICANT (S.D.S) TEST

Concentration (mg/L)	Rep	Day 0				Day 1			
		pH	DO	°C	Sal	pH	DO	°C	Sal
Control	1	7.93	9.4	15.4	31	7.75	5.0	NT	NT
	2					7.73	4.8	NT	NT
	3					7.69	4.8	NT	NT
1.6	1	7.94	9.4	15.2	31	7.62	4.0	NT	NT
	2					7.68	4.4	NT	NT
	3					7.70	4.4	NT	NT
3.1	1	7.95	9.4	15.2	31	7.59	4.1	NT	NT
	2					7.61	4.3	NT	NT
	3					7.64	4.4	NT	NT
6.25	1	7.95	9.4	15.2	31	7.42	2.1	NT	NT
	2					7.72	2.1	NT	NT
	3					7.75	2.2	NT	NT
12.5	1	7.96	9.4	15.2	31	7.42	2.0	NT	NT
	2					7.59	2.1	NT	NT
	3					7.56	2.1	NT	NT
25	1	7.96	9.4	15.2	31	7.40	2.0	NT	NT
	2					7.43	2.0	NT	NT
	3					7.48	2.0	NT	NT
Min		7.93	9.4	15.2	31	7.40	2.0		
Max		7.96	9.4	15.4	31	7.75	5.0		

Note: NT = Not taken.

APPENDIX TABLE 13

Citharichthys stigmaeus
SURVIVAL DATA
FOR REFERENCE TOXICANT (S.D.S.) TEST

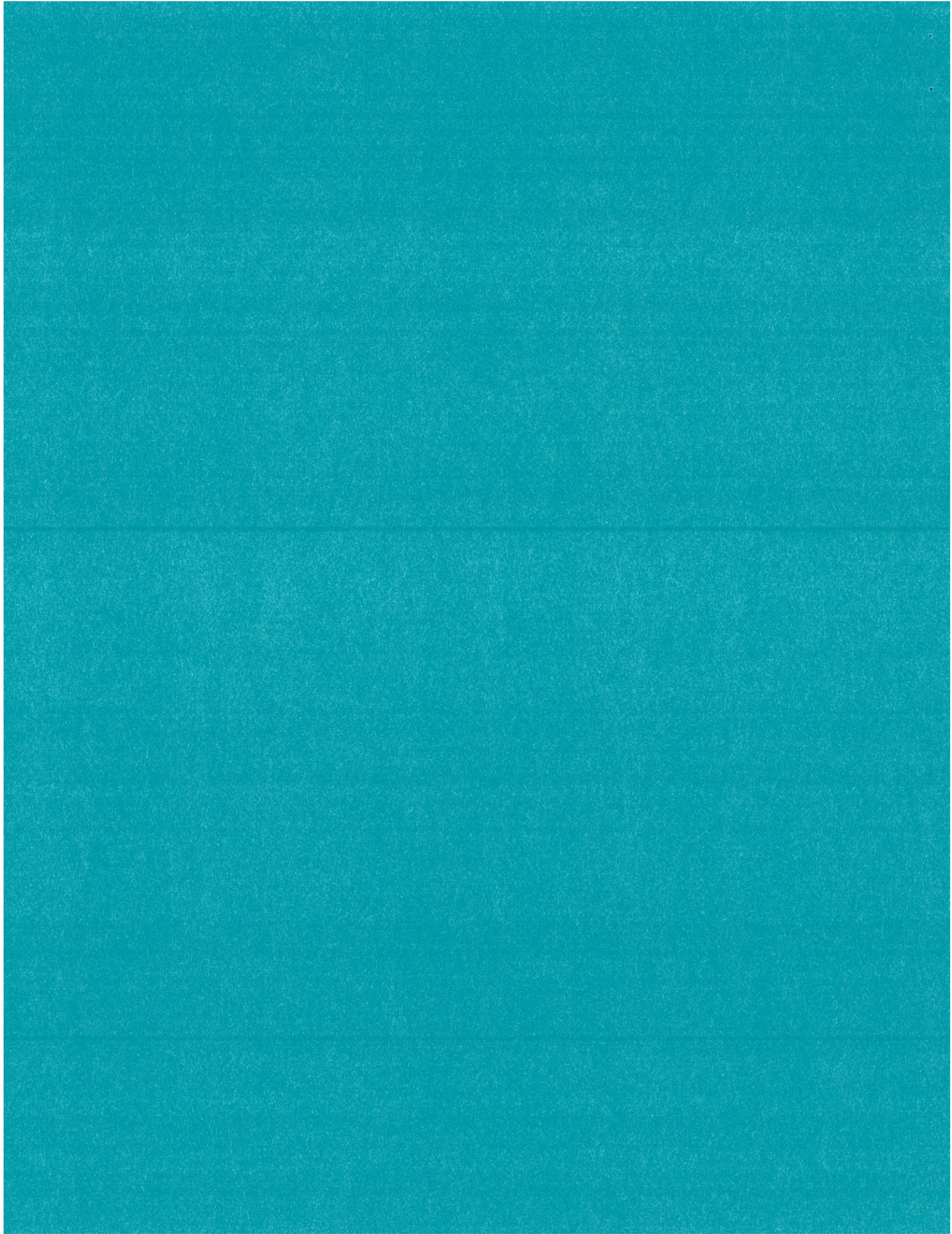
Concentration (mg/L)	Rep	Initial Added	Day 1	% Survival	Average % Survival
Control	1	6	6	100	100.0
	2	6	6	100	
	3	6	6	100	
1.6	1	6	6	100	100.0
	2	6	6	100	
	3	6	6	100	
3.1	1	6	5	83	83.3
	2	6	5	83	
	3	6	5	83	
6.25	1	6	0	0	0.0
	2	6	0	0	
	3	6	0	0	
12.5	1	6	0	0	0.0
	2	6	0	0	
	3	6	0	0	
25	1	6	0	0	0.0
	2	6	0	0	
	3	6	0	0	

ATTACHMENT 2

Standard Operating Procedures

High Strength Waste Sampling

for Bioassay Toxicity Tests



Standard Operating Procedures High Strength Waste Sampling for Bioassay Toxicity Tests

Introduction

Starkist Samoa, Inc. and VCS Samoa Packing are each required under their Ocean Disposal Dumping Permits to conduct definitive acute bioassays on their high strength waste (HSW) streams that are barged to sea for disposal at the permitted dump site. The following gives detailed procedures for collecting, preparing, and shipping samples for these analyses.

Each cannery is required to collect a composite sample of high strength waste while the waste is being transferred from the storage tanks to the barge. Currently a one gallon composite is required for the bioassay tests. The procedures described below are applicable to sampling at each of the canneries.

List of Equipment/Supplies

The following supplies will be required for collecting composite high strength waste samples and preparing them for delivery to the laboratories:

- Three (3) 1/2 to 1 gallon sampling containers
- One 1-gallon cubitainer or other appropriate container (container should be heavy-duty plastic with secure cap, do not ship samples in glass containers)
- Permanent marker for marking sample containers
- Cooler with ice (or refrigerator space) for storing sample
- Cooler for shipping samples (note: Cooler should be sized to hold sample(s) with sufficient room for ice.)
- Cubed ice (enough ice to fill airspace in cooler)
- Chain of Custody Forms (supplied by CH2M HILL or by laboratory conducting the analysis)

Sampling

The following describes the general sampling procedures:

- 1) **Collect "Grab" Samples.** Sampling should take place the day of or evening before the samples are shipped to the lab. Collect three 1/2 to 1-gallon grab samples from existing sampling ports in the storage tank transfer lines at the time waste is being transferred from the storage tanks to the barge. The samples should be collected at 10 minute intervals. Record the time each grab was taken. Store all samples in coolers on ice or in a

refrigerator at a temperature of approximately 4°C. Do **NOT** store samples in a freezer or using a method that would otherwise freeze the samples.

- 2) **Composite Samples.** Using a permanent marker, label the 1-gallon cubitainer with the following information:

- Facility samples were collected from
- Date
- Time each grab sample was collected

Combine the three grab samples by measuring 1/3 gallon of each into the 1-gallon cubitainer. Seal the sample container by placing plastic inside the cap and taping the cap down.

- 3) **Complete Chain of Custody Form.** One chain-of-custody form is required for each cooler in which samples are shipped. An example of a completed chain-of-custody form is included as Attachment A, along with a blank copy. Fill out the chain-of-custody form in triplicate or copy keeping one copy and sending two with the samples to the laboratory.

Shipping

The samples should be shipped the fastest way possible to:

Dr. Kurt Kline
Advanced Biological Testing, Inc.
3150 Paradise Drive, Building 50
Tiburon, CA 94920

Phone: (415) 435-7878; Fax: (415) 435-7882

The samples from each cannery can be shipped in separate coolers or in the same cooler. Place the composite sample into the cooler in which sample(s) is to be shipped. Ice, or an equivalent means such as chemical cold packs, should be used to fill in the empty space in the cooler and keep the sample(s) cold during shipping. Do not use dry ice to ship the sample. If cubed ice is used, precautions should be taken to prevent the melted ice from leaking out of the cooler during shipping. These include taping any drain plugs in the cooler shut with duct tape or strapping tape, and "double-bagging" the ice cubes in zip-lock bags, i.e. sealing the ice cubes in one bag, then sealing the bag containing ice in a second bag. As much air as possible should be removed from the bags prior to sealing. (Too much air inside the bags will expand during flight and pop the bag open).

The chain-of-custody form should be signed, placed in a zip-lock bag, and taped with duct tape to the inside of the cooler lid. The cooler should be taped securely with strapping tape or other strong packaging tape to prevent it from opening during shipping.

Attachment A
Example Chain-of-Custody Form

CH2M HILL Project #		Purchase Order #		LAB TEST CODES		SHADED AREA - FOR LAB USE ONLY	
00530702.DS.BT						Lab 1 # Lab 2 #	
Project Name OCEAN DUMPING PERMIT HIGH STRENGTH WASTE BIOASSAY				Quote # Kit Request #			
Company Name/CH2M HILL Office CH2M HILL / SFO				Project #			
Project Manager & Phone # Mr. [] STEVE COSTA Ms. [] Dr. [X] 510 251-2998		Report Copy to: SAME		No. of Samples COC Rev LIMS Ver Ack Gen			
Requested Completion Date: A.S.A.P		Sampling Requirements SDWA <input type="checkbox"/> NPDES <input type="checkbox"/> RCRA <input type="checkbox"/> OTHER <input checked="" type="checkbox"/> OCN		Page of			
Type C O M P O S I T E		Matrix S O I L		LAB 1 ID LAB 2 ID			
Sampling Date 10/18/00		CLIENT SAMPLE ID (9 CHARACTERS) V C S S A M O A I		REMARKS 1 - 1 gal cub. in ice ON ICE			
Sampled By & Title Barry Fowler / Barry Fowler		Date/Time 10/18/00		Relinquished By		Date/Time	
Received By		Date/Time		Relinquished By		Date/Time	
Received By		Date/Time		Relinquished By		Date/Time	
Received By		Date/Time		Shipped Via UPS BUS Fed-Ex Hand Other		Shipping #	
Work Authorized By		Date/Time		Remarks SAMPLE IS COMPOSITE OF 3 GRAB SAMPLES TAKEN AT 10 MINUTE INTERVALS			

CHAIN OF CUSTODY INSTRUCTIONS

CH2M HILL Project #: CH2M HILL project number to be charged for work.

Purchase Order #: Purchase order to be charged for work (OTC clients).

Project Name: Name of project which the samples support.

Company Name/CH2M HILL Office: Name of the company or CH2M HILL office requesting the work. Correspondence will be sent to the company address or CH2M HILL office.

Project Manager & Phone #: Name and phone number of person who receives the laboratory report and can be contacted if questions arise.

Report Copy To: Name and location of person to receive copy of laboratory report.

Requested Completion Date: When the report is required. Normal Turnaround Time (TAT) = 23 days (30 days for Hazwrad C/D or CLP). Faster TAT must be prearranged through Client Services.

Sampling Requirements: Program under which sampling and analysis are to be performed.

Sample Disposal: Indicate whether the samples are to be returned to the project manager or disposed by the laboratory.

Sampling: The date and time at which the sample was collected.

Type: Indicate the type of sample (composite or grab) collected.

Matrix: Indicate the sample matrix (water or soil)

Client Sample ID: Identifier assigned by the project to uniquely identify the samples (must not exceed nine (9) characters).

Number of Containers: The number of different containers for this line item or sample.

Analyses Requested: Use one column for each parameter or group of parameters. Specific method numbers, parameter list, and TIC's should be indicated.

For Lab Use Only: Do not mark in the shaded area.

Remarks: Record any comments about each sample on the same line as the sample description, e.g., "Wastewater contains VOC's." Known high concentrations should be noted.

Sampled by and Title: The person who took the sample signs this box and prints his/her name, title, date, and time when sampling was completed.

Relinquished By: The sampler signs this box and prints his/her name, date, and time when the samples are given to someone else.

Received By: The person who receives the samples signs here and prints his/her name, date, and time when the samples were accepted into his/her custody.

Sample Shipped Via: How the samples are being shipped to the laboratory, e.g., "Fed Ex."

Air Bill Number: The number on the shipping papers by which the package can be traced.

Work Authorized By: Printed name and signature of person authorizing the initiation of laboratory work.

Remarks: Record any comments regarding the samples as a whole. Additional parameters or special requirements should be indicated.

PROVISIONS

- 1. Authorization to Proceed**
Execution of this Agreement and Chain of Custody by the CLIENT will be authorization for CH2M HILL to proceed with the Laboratory work.
- 2. Compensation and Terms of Payment**
For services described on this Chain of Custody, CH2M HILL Quality Analytical Laboratories will be compensated based on a written quotation or the standard rates per analysis contained in our published price guide. Invoices will be issued by laboratories as services are completed. Invoices are due and payable upon receipt. Interest at the rate of 1-1/2 percent per month, or that permitted by law if lesser, may be charged on past due amounts starting 30 days after date of invoice. Payments will first be credited to interest and then to principal. The prices stated in a written quotation or on the price guide schedule do not include sales or other taxes. Such taxes, when applicable, will be added to the invoice. Unless otherwise specified, the minimum invoice is \$100.00. CH2M HILL Quality Analytical Laboratories reserve the right to change prices published in our price guide without notice.
- 3. Standard of Care**
The standard of care applied to our environmental laboratory services will be the degree of skill and diligence normally employed by laboratory industry personnel performing the same or similar service.
- 4. Warranty and Limitation of Liability**
CH2M HILL Quality Analytical Laboratories make no warranty, express or implied, and under no circumstances will be liable for any claims or damages except those resulting solely from their own or their employees' negligence. To the maximum extent permitted by law, our liability for damages will not exceed the compensation received by CH2M HILL Quality Analytical Laboratories under the project Agreement.
- 5. Severability and Survival**
If any of the provisions contained in this Agreement are held illegal, invalid or unenforceable, the enforceability of the remaining provisions shall not be impaired thereby. Limitations of liability and indemnities shall survive termination of this Agreement for any cause.
- 6. Asbestos or Hazardous Substances**
To the maximum extent permitted by law, the CLIENT will indemnify and defend CH2M HILL and its officers, employees, subconsultants, and agents from all claims, damages, losses, and expenses, including, but not limited to, direct, indirect, or consequential damages and attorney's fees in excess of the Limitation of Liability in Article 4 arising out of or relating to the presence, discharge, release, or escape of hazardous substances, contaminants, or asbestos on or from the Project.
- 7. Interpretation**
The limitations of liability and indemnities will apply whether CH2M HILL's liability arises under breach of contract or warranty; tort, including negligence (but not sole negligence); strict liability; statutory liability; or any other causes of action; and shall apply to CH2M HILL's officers, employees, and subcontractors. The professional services agreement will take precedence in the event there is a conflict with the agreement and chain-of-custody document.
- 8. Sample Disposal and Storage**
Disposal of hazardous waste samples is the responsibility of the CLIENT, unless disposal agreements are made. Hazardous waste samples will be returned 30 days after the submission of the analytical report, or disposed of at a rate of \$25 per sample. For large projects and upon special request, samples may be stored for longer than 30 days at a rate of \$5/month per sample.

CHAIN OF CUSTODY RECORD AND AGREEMENT TO PERFORM SERVICES

CH2M HILL Project # 0PE30702.DS.BT		Purchase Order #	
Project Name OCEAN DUMPING PERMIT HIGH STRENGTH WASTE BIOASSAY			
Company Name/CH2M HILL Office CH2M HILL /SFO			
Project Manager & Phone # Mr. [] STEVE COSTA Ms. [] Dr. [] 510 251-2888 X2251		Report Copy to: SAME	
Requested Completion Date: A.S.A.P.		Sampling Requirements SDWA <input type="checkbox"/> NPDES <input type="checkbox"/> RCRA <input type="checkbox"/> OTHER <input checked="" type="checkbox"/> OCN	
Sample Disposal: Dispose <input type="checkbox"/> Return <input type="checkbox"/>			
Type Matrix C G W S O R A O M A T I P B E R		CLIENT SAMPLE ID (9 CHARACTERS)	
Sampling Date Time 10/18 1000		X S T A R K I S T	
Date Time 10/18 1000		X S T A R K I S T	
Sampled By & Title Cliff Johnson		Date/Time 10/18 1000	
Received By Cliff Johnson		Date/Time 10/18 1000	
Received By		Date/Time	
Received By		Date/Time	
Work Authorized By		Remarks SAMPLE IS COMPOSITE OF 3 GRAB SAMPLES TAKEN AT 10 MINUTE INTERVALS	

Instructions and Agreement Provisions on Reverse Side

DISTRIBUTION: ORIGINAL - LAB, Yellow - LAB, Pink - Client
REV 11/92 FORM 340

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Report Copy To: Name and location of person to receive copy of laboratory report.

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CH2M HILL Quality Analytical Laboratories make no warranty, express or implied, and under no circumstances will be liable for any claims or damages except those resulting solely from their own or their employees' negligence. To the maximum extent permitted by law, our liability for damages will not exceed the compensation received by CH2M HILL Quality Analytical Laboratories under the project Agreement.
- 5. Severability and Survival**
If any of the provisions contained in this Agreement are held illegal, invalid or unenforceable, the enforceability of the remaining provisions shall not be impaired thereby. Limitations of liability and indemnities shall survive termination of this Agreement for any cause.
- 6. Asbestos or Hazardous Substances**
To the maximum extent permitted by law, the CLIENT will indemnify and defend CH2M HILL and its officers, employees, subconsultants, and agents from all claims, damages, losses, and expenses, including, but not limited to, direct, indirect, or consequential damages and attorney's fees in excess of the Limitation of Liability in Article 4 arising out of or relating to the presence, discharge, release, or escape of hazardous substances, contaminants, or asbestos on or from the Project.
- 7. Interpretation**
The limitations of liability and indemnities will apply whether CH2M HILL's liability arises under breach of contract or warranty; tort, including negligence (but not sole negligence); strict liability; statutory liability; or any other causes of action; and shall apply to CH2M HILL's officers, employees, and subcontractors. The professional services agreement will take precedence in the event there is a conflict with the agreement and chain-of-custody document.
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[illegible]

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Sampling: The date and time at which the sample was collected.

Type: Indicate the type of sample (composite or grab) collected.

Matrix: Indicate the sample matrix (water or soil).

Client Sample ID: Identifier assigned by the project to uniquely identify the samples (must not exceed nine (9) characters).

Number of Containers: The number of different containers for this line item or sample.

Analyses Requested: Use one column for each parameter or group of parameters. Specific method numbers, parameter list, and TIC's should be indicated.

For Lab Use Only: Do not mark in the shaded area.

Remarks: Record any comments about each sample on the same line as the sample description, e.g., "Wastewater contains VOC's." Known high concentrations should be noted.

Sampled by and Title: The person who took the sample signs this box and prints his/her name, title, date, and time when sampling was completed.

Relinquished By: The sampler signs this box and prints his/her name, date, and time when the samples are given to someone else.

Received By: The person who receives the samples signs here and prints his/her name, date, and time when the samples were accepted into his/her custody.

Sample Shipped Via: How the samples are being shipped to the laboratory, e.g., "Fed Ex."

Air Bill Number: The number on the shipping papers by which the package can be traced.

Work Authorized By: Printed name and signature of person authorizing the initiation of laboratory work.

Remarks: Record any comments regarding the samples as a whole. Additional parameters or special requirements should be indicated.

PROVISIONS

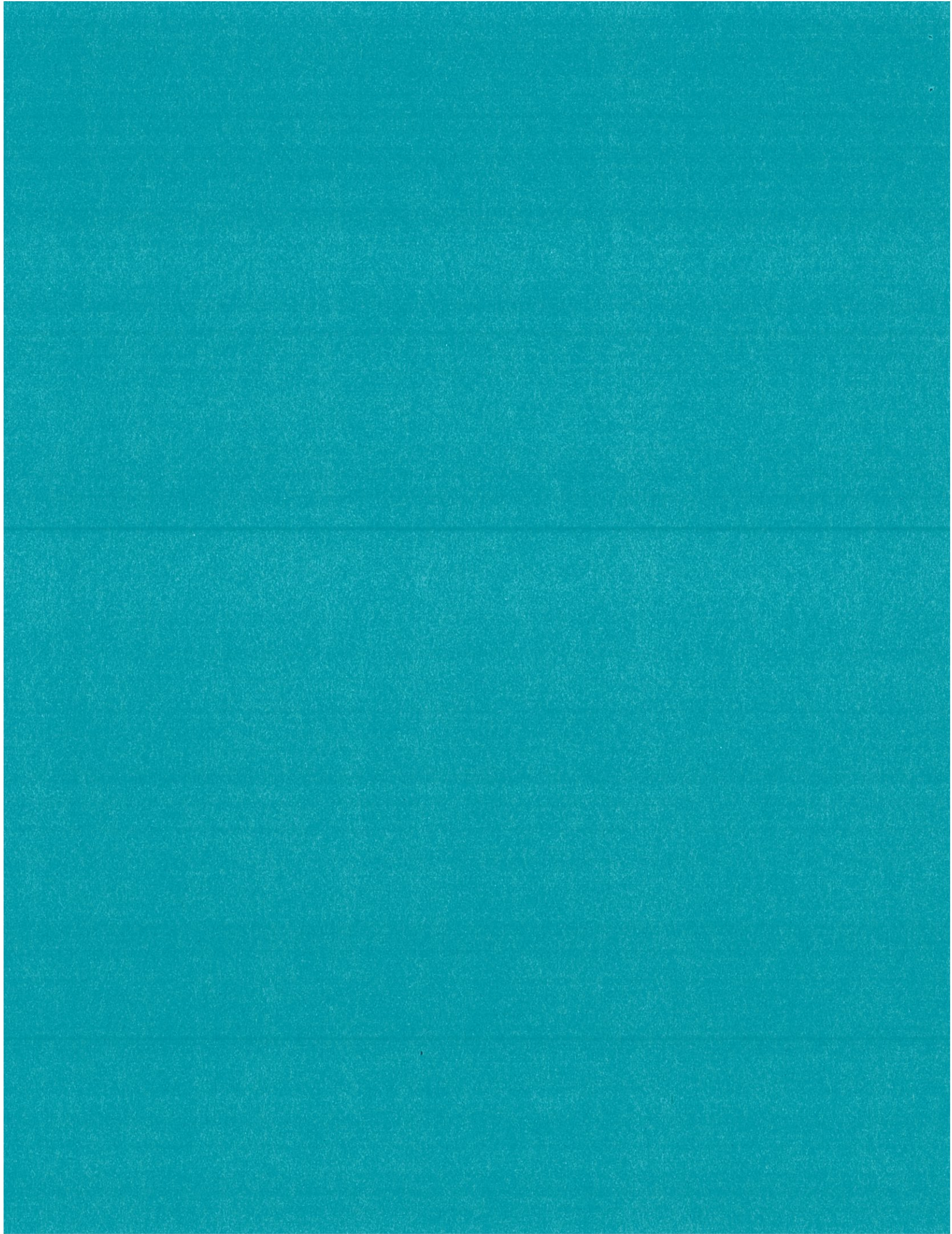
- 1. Authorization to Proceed**
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- 2. Compensation and Terms of Payment**
For services described on this Chain of Custody, CH2M HILL Quality Analytical Laboratories will be compensated based on a written quotation or the standard rates per analysis contained in our published price guide. Invoices will be issued by laboratories as services are completed. Invoices are due and payable upon receipt. Interest at the rate of 1-1/2 percent per month, or that permitted by law if lesser, may be charged on past due amounts starting 30 days after date of invoice. Payments will first be credited to interest and then to principal. The prices stated in a written quotation or on the price guide schedule do not include sales or other taxes. Such taxes, when applicable, will be added to the invoice. Unless otherwise specified, the minimum invoice is \$100.00. CH2M HILL Quality Analytical Laboratories reserve the right to change prices published in our price guide without notice.
- 3. Standard of Care**
The standard of care applied to our environmental laboratory services will be the degree of skill and diligence normally employed by laboratory industry personnel performing the same or similar service.
- 4. Warranty and Limitation of Liability**
CH2M HILL Quality Analytical Laboratories make no warranty, express or implied, and under no circumstances will be liable for any claims or damages except those resulting solely from their own or their employees' negligence. To the maximum extent permitted by law, our liability for damages will not exceed the compensation received by CH2M HILL Quality Analytical Laboratories under the project Agreement.
- 5. Severability and Survival**
If any of the provisions contained in this Agreement are held illegal, invalid or unenforceable, the enforceability of the remaining provisions shall not be impaired thereby. Limitations of liability and indemnities shall survive termination of this Agreement for any cause.
- 6. Asbestos or Hazardous Substances**
To the maximum extent permitted by law, the CLIENT will indemnify and defend CH2M HILL and its officers, employees, subconsultants, and agents from all claims, damages, losses, and expenses, including, but not limited to, direct, indirect, or consequential damages and attorney's fees in excess of the Limitation of Liability in Article 4 arising out of or relating to the presence, discharge, release, or escape of hazardous substances, contaminants, or asbestos on or from the Project.
- 7. Interpretation**
The limitations of liability and indemnities will apply whether CH2M HILL's liability arises under breach of contract or warranty; tort, including negligence (but not sole negligence); strict liability; statutory liability; or any other causes of action; and shall apply to CH2M HILL's officers, employees, and subcontractors. The professional services agreement will take precedence in the event there is a conflict with the agreement and chain-of-custody document.
- 8. Sample Disposal and Storage**
Disposal of hazardous waste samples is the responsibility of the CLIENT, unless disposal agreements are made. Hazardous waste samples will be returned 30 days after the submission of the analytical report, or disposed of at a rate of \$25 per sample. For large projects and upon special request, samples may be stored for longer than 30 days at a rate of \$5/month per sample.

ATTACHMENT 3

1 July 1994 Memo to EPA

Recommending Changes to the High Strength Waste

Bioassay Testing Protocols



MEMORANDUM

CH2M HILL

TO: Pat Young/USEPA

COPIES: Amy Wagner/USEPA (w/ attachments)
Kurt Kline/ABT (w/o attachments)

FROM: Steve Costa/CH2M HILL/SFO
Don Kingery/CH2M HILL/SFO

DATE: July 1, 1994

SUBJECT: Bioassay Testing of Starkist Samoa, Inc. and VCS Samoa Packing High Strength Waste

PROJECT: OPE030702.EL.R2

High strength waste (HSW) bioassays are required by Special Condition 3.3.5 of Starkist Samoa's and VCS Samoa Packing's ocean dumping permits. The results of the tests are presented in the attached: "*Results of a Bioassay Conducted on Two High Strength Waste Samples from the Van Camp and Starkist Tuna Canneries in American Samoa*" prepared by Advanced Biological Testing Inc., Tiburon, California.

Acute effluent bioassays were conducted on *Mysidopsis bahia* (mysid shrimp) juveniles, *Mytilus edulis* (blue mussel) larvae, *Strongylocentrotus purpuratus* (purple sea urchin) larvae, and *Citharichthys stigmaeus* (speckled sanddab) juveniles using HSW collected separately from the Starkist Samoa and VCS Samoa Packing canneries in Pago Pago Harbor, American Samoa. The results of these bioassays are summarized in the table below.

Based on the results of the bioassays, we recommend the following changes to the HSW bioassay protocol:

Reduce the upper end of the HSW concentration series for all bioassays to a maximum of 3.0%. The results of the bioassay tests give a better understanding of the test concentrations needed. No additional information is required at concentrations greater than 3.0%. Reducing the maximum concentrations will reduce the amount of HSW that needs to be sampled and shipped. We recommend a series of concentrations for the bioassays of 3.0%, 1.5%, 0.8%, 0.2%, 0.1%, and 0.05%.

Continue running bioassays with *Mytilus edulis* while monitoring the effects of aeration on organism mortality but drop the use of *Strongylocentrotus purpuratus* larvae as test organisms for the HSW. This recommendation is made for the following reasons:

- Special Condition 3.3.5 of the permits requires only three organisms be tested; one organism each out of three specified groups. *Mysidopsis bahia* and

MEMORANDUM

Page 2

July 1, 1994

OPE030702.EL.R2

Citharichthys stigmaeus satisfy the requirements for Groups 2 and 3. Group 1 contains larval stages of both bivalves and echinoderms and running just *Mytilus edulis* should satisfy this requirement.

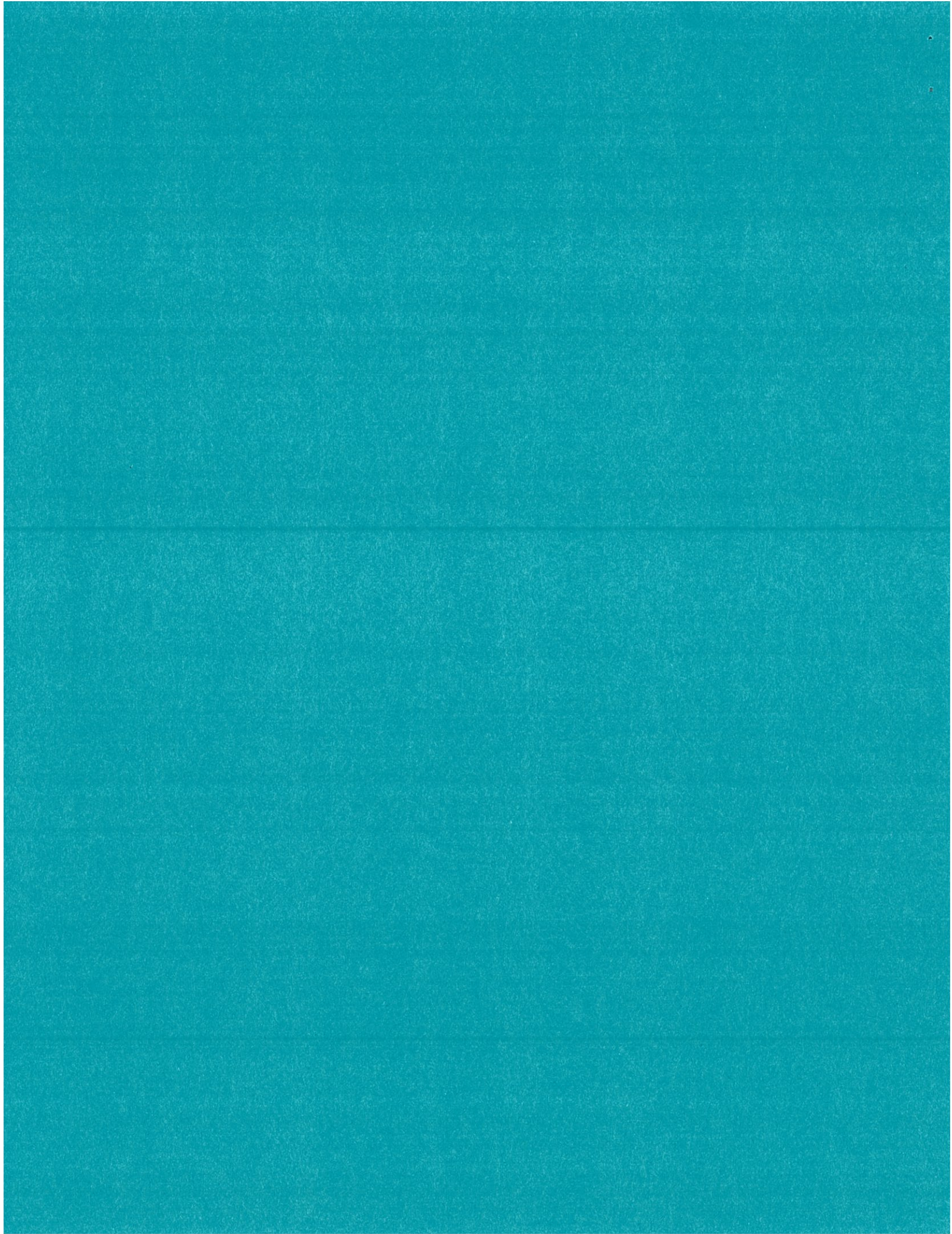
- Because of the high oxygen demand of the effluent, all test containers required aeration throughout the tests to maintain adequate oxygen concentrations for the test organisms. Aerating the chambers using *Mytilus edulis* and *Strongylocentrotus purpuratus* larvae as bioassay test organisms gives problematic results. Aeration is standard protocol for bioassays on fish and invertebrates when oxygen levels fall below 40% of saturation, but is not standard protocol for bioassays on larval bivalves and echinoderms. The effects of aerating the water on the survival of these organisms is not known. Because the *Mytilus edulis* bioassays are only run for two days (vs. four for the *Strongylocentrotus purpuratus*) the organisms are exposed for half the time and the effects of aeration may be reduced.
- The mortality of the control group was substantial for the echinoderms and is unacceptable according to protocol. The cause of the high mortality in the control is not known at this time.

Please review the above recommendations. We suggest Amy Wagner contact Kurt Kline, Advanced Biological Testing Inc., directly at (415)435-7878 to discuss any comments you have on the bioassay protocols.

Summary of High Strength Waste Bioassay Results.				
Test Organism	Starkist Samoa		VCS Samoa Packing	
	LC ₅₀	NOEC/IC ₅₀ ¹	LC ₅₀	NOEC/IC ₅₀ ¹
<i>Citharichthys stigmaeus</i> (sanddab)	0.27%	0.2%	0.59%	0.4%
<i>Mysidopsis bahia</i> (mysid shrimp)	0.12%	0.05%	0.59%	0.05%
<i>Mytilus edulis</i> (blue mussel)	> 1.2%	< 0.08%	> 1.2%	< 0.08
<i>Strongylocentrotus purpuratus</i> ² (urchin)	> 1.2%	< 0.08%	> 1.2%	0.1%
¹ NOEC reported for the juvenile sanddabs and mysid shrimp, IC ₅₀ reported for the mussel and urchin larvae.				
² Control survival of 64.4% is unacceptable according to protocol.				

ATTACHMENT 4

**29 August 1994 Response From EPA
Containing Acceptable Changes to the
High Strength Waste Bioassay Testing Protocols**





UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX
75 Hawthorne Street
San Francisco, CA 94105

August 29, 1994

Steven L. Costa
Project Manager
CH2M Hill
P.O. Box 12681
Oakland, CA 94604-2681

Re: Comments on Bioassay Testing of Ocean Disposed High-Strength
Waste of StarKist Samoa, Inc. and VCS Samoa Packing Company

Dear Steve:

We have reviewed the report of June 29, 1994 for the first of three rounds of bioassays of high-strength waste, as required by the canneries' ocean disposal permits. The report is based on two sampling events: the first was collected on February 16, 1994; and, a second sample was required and tested in March 1994, due to test failure of the echinoderms in the first sample. Your proposed changes to the study methods, as outlined in your memo of July 1, 1994, are acceptable. Enclosed is a memo from Amy Wagner of EPA's Laboratory Support Section, detailing the acceptable changes. Please call Amy at (510) 412-2329 if you have any questions on her comments.

We note that the second and third rounds of testing were scheduled for May and August 1994, and we would like to know if these tests were conducted as scheduled and, if not, the rescheduled dates, and when we can anticipate the reports on these bioassays. Please relay this information to Pat Young, American Samoa Program Manager, or if you have any questions, call her at (415) 744-1594.

Sincerely,

M. J. Lee
Norman L. Lovelace, Chief
Office of Pacific Island and Native
American Programs (E-4)

Enclosure

cc: Jim Cox, Van Camp Seafood Company
Norman Wei, StarKist Seafood Company
Tony Tausaga, American Samoa EPA
Sheila Wiegman, American Samoa EPA
Allan Ota, W-3-3
Amy Wagner, P-3-1



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX LABORATORY
1337 S. 46TH STREET BLDG 201
RICHMOND, CA 94804-4698

AUG 29 1994

MEMORANDUM

SUBJECT: Review of Bioassay Testing of Starkist, Samoa, Inc. and VCS Samoa Packing High Strength

FROM: *AW*
Amy Wagner
Laboratory Section (P-3-1)

THRU: *Brenda Bettencourt*
Brenda Bettencourt, Chief
Laboratory Section (P-3-1)

TO: Pat Young
OPINAP (E-4)

Allan Ota
Wetlands and Sediment Management Section (W-3-3)

At your request, I have reviewed "Results of a Bioassay Conducted on Two High Strength Waste Samples from the Van Camp and Starkist Tuna Canneries in American Samoa." The following recommendations are based on the results of the first round of testing.

1. p. 11. The salinity of the *Mysidopsis bahia* tests were 25 ppt, presumably based on the salinity of the shipping water. An effort should be made to find a supplier that raises mysids in a salinity closer to that of the discharge site, between 30-35 ppt.
2. Appendix, p. 1. It is recommended that the water quality measurements pH, dissolved oxygen, and initial salinity be measured for all samples upon receipt.

3. Appendix, Table 10. The salinities of 26-28 ppt most likely caused the high mortality in controls with the sea urchin toxicity test. If necessary, brine adjustments should be used to increase the salinity of test samples to the test method requirements of 30 ± 2 ppt.
4. To reduce salinity elevation throughout the tests, an attempt should be made to cover test containers to reduce evaporation.

Based on the results of these tests, the following changes in the bioassay methods recommended by CH2M Hill in the cover memo are acceptable.

1. The series of the concentrations for toxicity tests can be reduced to 2.0%, 1.0%, 0.5%, 0.25%, 0.125%, and 0.0625% instead of the suggested series.
2. *Mytilus edulis* can be used instead of *Strongylocentrotus purpuratus* as the third test organism. The oyster *Crassostrea virginica* may be substituted for the mussel test during the months when mussels cannot be spawned.
3. Aeration should be provided in the mussel test containers due to high biological oxygen demand of the effluent. In addition to a control with aeration, a control without aeration should be run. A t-test should be used to determine if there is any significant effect of aeration.

Any questions on the comments can be addressed to me at (510) 412-2329.

cc: Jeff Rosenbloom, Chief
Wetlands and Sediment Management Section (W-3-3)

